BIOCHEMISTRY

THIRD EDITION

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W. H. FREEMAN AND COMPANY / NEW YORK

Library of Congress Cataloging-in-Publication Data

Stryer, Lubert.
Biochemistry.

Includes index.
1. Biochemistry. I. Title.
QP514.2.S66 1988 574.19'2 87-36486
ISBN 0-7167-1843-X
ISBN 0-7167-1920-7 (international student ed.)

Printed in the United States of America

5 6 7 8 9 0 RRD 9 9 8 7 6 5 4 3 2 1 0

Restriction enzymes are used to cleave DNA molecules into specific fragments that are more readily analyzed and manipulated than the parent molecule. For example, the 5.1-kilobase (kb) circular duplex DNA of the tumor-producing SV40 virus is cleaved at one site by EcoRI, four sites by HpaI, and eleven sites by HindIII. A piece of DNA produced by the action of one restriction enzyme can be specifically cleaved into smaller fragments by another restriction enzyme. The pattern of such fragments can serve as a *fingerprint* of a DNA molecule, as will be discussed shortly. Indeed, complex chromosomes containing hundreds of millions of base pairs can be mapped by using a series of restriction enzymes.

Kilobase (kb)—
A unit of length equal to 1000 base pairs of a double-stranded nucleic acid molecule (or 1000 bases of a single-stranded molecule).
One kilobase of double-stranded DNA has a contour length of 0.34 μm and a mass of about 660 kd.

RESTRICTION FRAGMENTS CAN BE SEPARATED BY GEL ELECTROPHORESIS AND VISUALIZED

Small differences between related DNA molecules can be readily detected because their restriction fragments can be separated and displayed by gel electrophoresis. In many types of gels, the electrophoretic mobility of a DNA fragment is inversely proportional to the logarithm of the number of base pairs up to a certain limit. Polyacrylamide gels are used to separate fragments containing up to about 1000 base pairs, whereas more porous agarose gels are used to resolve mixtures of larger fragments (up to about 20 kb). An important feature of these gels is their high resolving power. In certain kinds of gels, fragments differing in length by just one nucleotide out of several hundred can be distinguished. Moreover, entire chromosomes containing millions of nucleotides can now be separated on agarose gels by applying pulsed electric fields in different directions (p. 46). Bands or spots of radioactive DNA in gels can be visualized by autoradiography. Alternatively, a gel can be stained with ethidium bromide, which fluoresces an intense orange when it has been bound to double-helical DNA. A band containing only 50 ng of DNA can readily be seen (Figure 6-3).

A restriction fragment containing a specific base sequence can be identified by hybridizing it with a labeled complementary DNA strand (Figure 6-4). A mixture of restriction fragments is separated by electro-

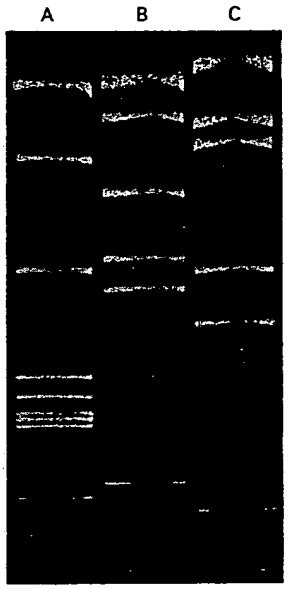

Figure 6-3
Gel electrophoresis pattern showing the fragments produced by cleaving SV40 DNA with each of three restriction enzymes. These fragments were made fluorescent by staining the gel with ethidium bromide. [Courtesy of Dr. Jeffrey Sklar.]

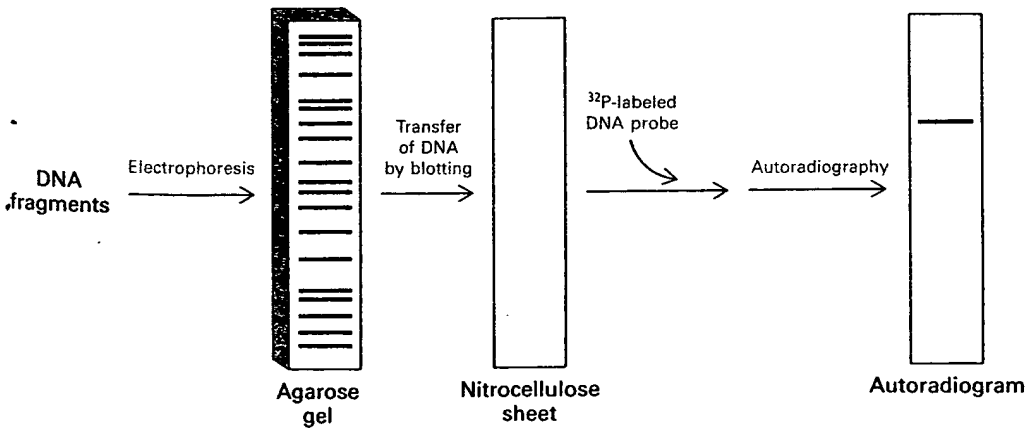

Figure 6-4
Southern blotting. A DNA fragment containing a specific sequence can be identified by separating a mixture of fragments by electrophoresis, transferring them to nitrocellulose, and hybridizing with a ^{32}P-labeled probe complementary to the sequence. The fragment containing the sequence is then visualized by autoradiography.